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Antiproliferative stent coatings: Taxol and related compounds

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The implantation of stents can prevent vessels from post interventional elastic recoil and appears to limit adverse remodelling. In order to inhibit in-stent restenosis, an additional release of antiproliferative agents from the stent itself might lead to a synergistic reduction of lumen renarrowing. Paclitaxel (Taxol®) is a microtubule-stabilizing agent with potent antiproliferative activity. Unlike other antimitotic agents of the colchicine type, it shifts the microtubule equilibrium towards assembly, leading to reduced proliferation, migration and signal transduction. Moreover, important biological processes, such as the activation of some protein kinases, are associated with microtubule depolymerization and are therefore inhibited by paclitaxel. Several experimental *in vitro* and *in vivo* studies using local paclitaxel delivery to inhibit proliferation and lumen renarrowing have been performed already—with very encouraging results.

Key words: paclitaxel, Taxol, stents, local delivery, smooth muscle, restenosis

Introduction

Even with the increasing number of stent implantations to treat coronary lesions, the restenosis problem remains far from being resolved. However, at least theoretically, the treatment strategy for the prevention of an in-stent restenosis has become apparent. The implantation of stents can prevent elastic recoiling and appears to limit adverse remodelling, both important components of the restenosis process. Theoretically, an additional release of antiproliferative agents from the stent itself could lead to a synergistic reduction of lumen renarrowing, since all the main factors involved in the restenosis process would be addressed with one intervention.

Numerous pharmacological agents with antiproliferative properties have been tested for their potential to inhibit restenosis. Although initially promising, the results in clinical use have mostly been disappointing so far [1]. Even potent antimitotic compounds like methotrexate and colchicine have failed to inhibit smooth muscle cell proliferation and intimal thickening *in vivo* [2, 3].

Paclitaxel, however, exerts its pharmacological effects through an exceptional mechanism. It causes the formation of numerous decentralized and unorganized microtubules and enhances the assembly of extraordinarily stable microtubules, thereby inducing cellular modifications that result in reduced proliferation, migration and signal transduction [4–6]. Unlike other antiproliferative agents of the colchicine type, which inhibit microtubule assembly, it shifts the microtubule equilibrium towards microtubule assembly. Since several important biological processes (like the activation of protein kinases) are associated with microtubule depolymerization, these processes are therefore inhibited by paclitaxel. Moreover, paclitaxel reduces the growth-factor-stimulated release of transcription factors (such as nuclear factor-κB) and therefore influences the expression of proto-oncogenes at different stages [7–9].

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Paclitaxel (Taxol®)

Paclitaxel (Taxol®) was originally isolated from the bark of the Pacific Yew Taxus brevifolia. It is a diterpenoid with a characteristic taxane-skeleton of 20 carbon atoms and has a molecular weight of 853.9 Dalton. Although the toxicity of extracts from the yew has been known for ages (being 'successfully' used for murder and suicide), it was not until 1964 that the National Cancer Institute in the USA started a screening program for antineoplastic substances and launched the unique pharmacological career of Taxol®. The 'success story' of Taxol® in the treatment of human malignancies is unparalleled and has even interfered with our daily lives in the 1980s, when medicine and ecology came into conflict: initially, 10–15000 kg of bark were needed to extract 1 kg of paclitaxel, which was a serious

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threat to the Pacific Yew in North America [4–6, 10]. Today, synthetically-produced Taxol® has become a standard medication in oncology.

Canizares *et al.* recently reported paclitaxel to have potent inhibitory effects on platelet aggregation. Since the platelet activation process goes along with a morphological transformation of the platelet, it could be effectively inhibited by paclitaxel due to its microtubule-stabilizing potential [11]. This finding might be of important clinical relevance for an additional prevention of stent thrombosis and for inhibition of very early stages of the restenosis process.

Paclitaxel is highly lipophilic, which facilitates a rapid cellular uptake and has a long-lasting effect in the cell due to the structural alteration of the cytoskeleton [12, 13]. These pharmacological properties make paclitaxel a very interesting compound for local drug therapy.

Experiences with paclitaxel

Sollot *et al.* were the first to report on antiproliferative effects of paclitaxel on rat smooth muscle cells *in vitro*, as well as *in vivo* [14]. In our own work, we initially studied the *in vitro* effects of paclitaxel on proliferation and migration of human arterial smooth muscle cells (haSMCs). Both monocultures of haSMCs and cocultures with human arterial endothelial cells were used in that study. Cell growth in the absence or presence of growth factors was determined after 4, 8 and 14 days. We were able to demonstrate that even after a short single dose application (10 min) on human smooth muscle cell cultures, paclitaxel (0.1–10 µmol/l) exerted a sustained antiproliferative effect over a period of 14 days without showing rebound or cytotoxic effects [15]. Higher doses of paclitaxel were needed to inhibit endothelial cell growth in a similar way, a finding consistent with the *in vivo* experiments. In a subsequent *in vivo* study, the potential of locally delivered-paclitaxel to prevent restenosis was assessed in an experimental rabbit angioplasty model. After the induction of a defined plaque in the right carotid arteries of New Zealand rabbits by electrical stimulation, the animals underwent balloon dilatation and subsequent local paclitaxel delivery (10 ml, 10 µmol/l) with a double balloon. The extent of stenosis in paclitaxel-treated animals was significantly reduced compared to balloon-dilated control animals 2 months after intervention (20.5 ± 5.3 vs $43.1 \pm 17.3\%$, $p \leq 0.003$). With tubulin staining and electron microscopy, we identified paclitaxel-induced changes in microtubule assembly. Similar to other cytoskeleton-affecting drugs, paclitaxel also altered vasocontractile function. In additional experiments, the incubation of vascular segments in paclitaxel (10 min, 10 µmol/l) led to a decrease in relative contraction force to 22.8% compared to control segments [16].

These encouraging results favoured the use of paclitaxel for the prevention of in-stent restenosis to address the predominantly proliferative restenotic process after stent implantation. First, delivery efficiency with stent implantation prior to or following local paclitaxel delivery needed to be determined. Radiolabelled (^{3}H) preparations (5 ml) of paclitaxel were injected into the left anterior descending artery of freshly explanted porcine hearts with the Infusasleeve II. It showed that delivery efficiency proved to remain unchanged at any given application pressure, whether stenting and local drug delivery (with prior stenting or stenting following delivery) or local paclitaxel delivery only was performed. It could be demonstrated that delivery efficiency was around 2% in all these experiments (differences non statistically significant) [17]. In a follow-up study we analysed the effects of locally-delivered paclitaxel following stent implantation in native coronary arteries of 21 domestic pigs. Paclitaxel (5 ml, 10 µmol/l) was delivered with the Infusasleeve II catheter following stenting of the left anterior descending artery. After 4 weeks, the vessels were excised. In this study, however, no relevant differences between treatment group and controls were identified. Thus, paclitaxel was not able to reduce neointima formation in native pig coronary artefies, probably due to extensive injury and overdistension of the vessel after stenting [18]. It may well be that a sustained paclitaxel release from a stent coating is the superior concept when combining stenting and local paclitaxel therapy. One can speculate, however, whether a constant paclitaxel release for weeks will also lead to increased inflammation, necrosis and cell death around the stent struts. Haehnel *et al.* performed experiments with paclitaxel released from a biodegradable stent coating in a cell culture. Paclitaxel was applied to polylactic acid as a drug carrier and was released from there for several days, leading to a dose-dependent growth inhibition of vascular smooth muscle cells in the immediate proximity of the drug. Long-term animal experiments are under way [19].

Side-effects

In addition to the possible local complications after stent implantation with paclitaxel coating, concern was expressed that adverse systemic effects might result from a local paclitaxel delivery. In fact, several toxic effects are known for paclitaxel in high-dose chemotherapy for malignant disease. The principal side-effects are haematological toxicity (neutropenia), neurotoxicity (peripheral neuropathy), and hypersensitivity reactions. Cardiac disturbances (mostly transient asymptomatic bradycardia) have also been reported [6, 20–23]. However, the plasma levels of paclitaxel in these patients (undergoing high-dose chemotherapy) were 100–1000-fold higher over a longer time period than the plasma levels that would result from a local infusion of paclitaxel. With a detectability limit of about 50 nmol/l, local paclitaxel delivery in doses used thus far in the

experimental studies would not even result in measurable plasma levels in humans [24].

Future developments

The urgent need for more paclitaxel led to a worldwide struggle among renowned chemists for a total synthesis of paclitaxel. It was not until 1994 that this goal was achieved by Holton *et al.* and Nicolaou *et al.*, who published their (different) strategies for a total synthesis [25, 26]. Now these experiences are also used to create new and more potent derivatives of paclitaxel around its characteristic taxane-structure. One very successful example is docetaxel (Taxotere®), which was developed by Potier *et al.* In various pharmacological tests, Taxotere® has shown to be more effective than Taxol® and better tolerated by the patients [3, 22]. In the future, we will certainly see more compounds evolve from this search for better efficacy and fewer side-effects.

Conclusion

The concept of loading a stent with paclitaxel to inhibit in-stent stenosis is fascinating. Paclitaxel appears to be a very promising compound for local drug delivery because of its lipophilic character, which facilitates a rapid cellular uptake, and its long-lasting and potent action. It has proved to be very effective on smooth muscle cells and platelets, whereas higher doses are needed to inhibit endothelial cells in a similar way. Together with the inherent effects of the stent itself on recoil and remodelling, a combination of stents and the antiproliferative paclitaxel should thus affect all the major components involved in the restenotic process. Large animal studies to test this hypothesis are now under way.

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